DIMA BIOTECH
Recombinant mAbs and proteins

Risk-free Lead Antibody Discovery

Over 5000 Pre-validated Monoclonal Antibodies for 300+ Drug Targets

Dedicate on Drug Targets,
Specialize in recombinant mAb development

Challenges and opportunities for antibody drug discovery

Monoclonal antibodies (mAbs) are the fastest growing class of drugs over the past decade. With recent scientific discovery on new mechanisms of immune-oncology and progress of new technologies in mAbs development, more and more antibody drug candidates are on the horizon. Antibody drug occupied almost 20% of all FDA approved medicines during the past few years. Most of them are for treatment of cancer patients. The characteristics of antibody drugs, e.g., high specificities and low level side effects, make them the most popular drug category in medical market. In 2022, two antibody drugs, Humira and Keytruda are within the top 3 sales of drugs, only after mRNA vaccine (Comirnaty). Until now, FDA has approved more than 100 antibody drugs with the size of market share over 1500 billion dollars.

Antibody drugs normally target the membrane proteins on the cell surface that are related to human diseases. The drug formats evolved from original simple monoclonal antibody to various innovative formats, e.g., bi-specific antibody, antibody drug conjugates (ADC) and Nanobodies. The market for therapeutic antibody drugs has experienced explosive growth as new drugs have been approved for different human diseases, including cancers, autoimmune, metabolic and infectious diseases.

Formats of Antibody Drugs:

- Fully human or humanized mAbs: Fully human or humanized antibodies greatly reduce immune side effects caused by heterologous antibodies to human body, thereby improving the therapeutic effect. They are the most common format of antibody drugs.
- Antibody–drug conjugate (ADC) is typically composed of a monoclonal antibody covalently attached to a cytotoxic drug via a chemical linker. It combines both the advantages of highly specific targeting ability and highly potent killing effect to achieve accurate and efficient elimination of cancer cells, which has become one of the hotspots for research and development of anticancer drugs.
- Bispecific or multi-specific antibody drug is a rising star in antibody drugs. Two or multiple antibody fragments against different antigens are fused through antibody engineering, which improves the killing effect of cancer cells through multi-target, multi-pathway inhibitions. So far, FDA has approved 2 bispecific antibodies.

- Nanobodies have many unique properties such as small size, good solubilities and stabilities, quick clearance and strong tissue penetration abilities, which make them promising tools for diagnosis and therapy of diseases. They can improve the efficacy of antibody drug therapy through the lesion-specific delivery of drugs and effector domains.
- CAR-T cell therapy is a new direction for antibody drug research and development. The variable regions of antibody heavy and light chains are constructed into a CAR structure and transduced into the patient's T cells as a single-chain fragment (scFv). The engineered CAR-T cells can be used to kill tumor cells in vivo.

Antibody drug development is like a marathon, from initial target research to the development of lead antibody molecules, preclinical functional and toxicity research, to the later clinical I/II/III experimental stage, and finally NDA declaration, which requires a lot of money and time, with a high degree of uncertainty and risk (Figure 1).

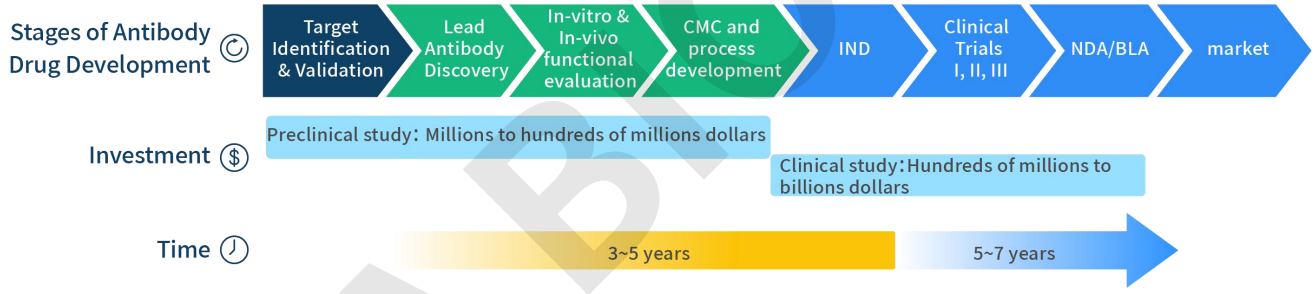


Figure 1: This schematic shows investment and time required for the preclinical and clinical stages of antibody drug development. The process of antibody drug development is very costly and lengthy. A good lead mAb molecule can be the entry key to the process.

Antibody drug development has been focused on a few popular targets, e.g., PD-1/PD-L1, HER2 for a long time. However, the studies of other targets have never stopped. Out of all FDA approved antibody drugs in 2021, most of them are First in Class (FIC) antibody drugs except Dostarlimab for PD-1. This highlights a new trend of antibody drug development, e.g., new targets and new formats. Currently there are approximately 400~500 targets identified as tumor targets. As more and more innovative targets are discovered, it is difficult to rely solely on company's own preclinical R&D capabilities or its CRO partners to make early antibody molecule discovery. Both have their own pros and cons, as summarized in table 1. Due to the complexity and unpredictability of therapeutic lead mAb discovery process, there will be no guarantees of success at this stage, which impose a bottleneck for the current antibody drug development.

Table 1: Pros and Cons of different strategies for lead antibody discovery

	Internal R&D team	CRO companies
Pros	Manage internally	Flexibility
	easy communications	small or no equipment investment
Cons	Limitations on internal R&D capacity and efficacy	Upfront payment with little guarantees
	Hard to control development timeline	Hard to control development timeline
	Initial big investment on equipment and reagents	Extensive management cost
	High risk on project, No guarantees	High risk on project, No guarantees

DIMA's Solutions for Lead Antibody Discovery

All Druggable Targets (ADT) Program

To help Biopharma accelerate its pace on pre-clinical antibody drug lead selection and optimization, DIMA, equipped with its proprietary single B cell discovery platform, launched an "All Druggable Targets (ADT)" lead discovery program. The goal is to provide on-shelf pre-stocked and pre-validated hit-to-lead stage antibody molecules for all druggable targets. Compared to traditional in-house R&D or CRO models, DIMA lead antibody molecules provide an on-shelf solution for pharmaceutical companies. Our **zero-waiting, zero-risk** business model allows pharmaceutical companies to effectively avoid unnecessary risks and financial investments during early drug discovery stage. The complete list of our available targets can be found on our website (www.dimabio.com/lead-antibody-molecule).

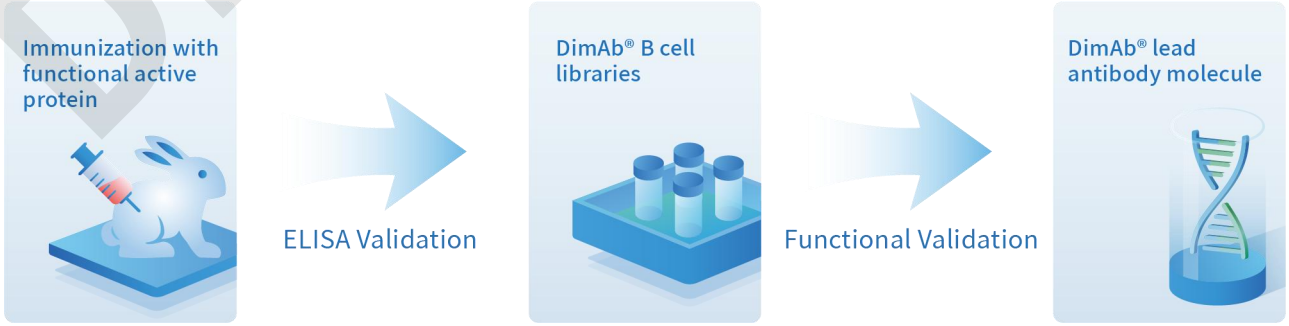


Figure 2: DIMA Biotech ADT Lead Discovery Program

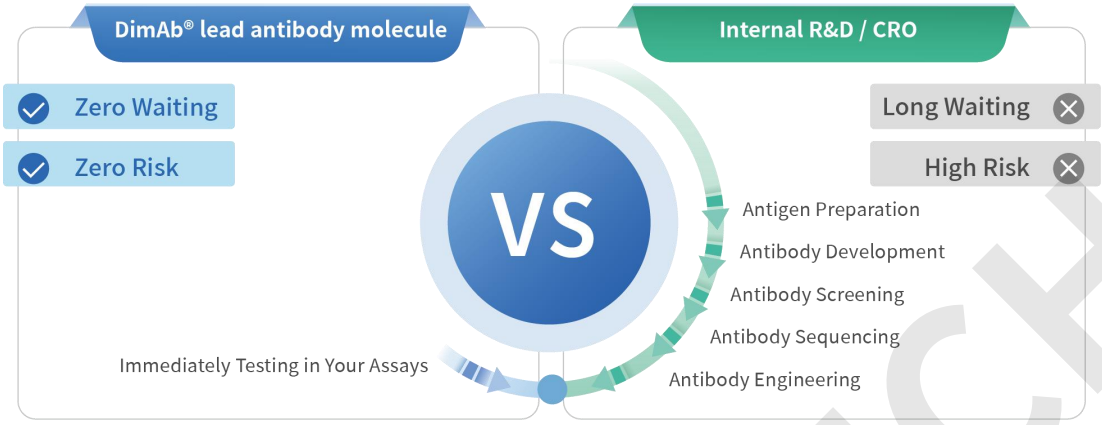


Figure 3: Comparison of DimAb lead discovery program and traditional lead discovery strategies

After in-vitro and in-vivo screenings of pre-selected mAbs, one or several druggable mAbs can be picked up for the downstream clinical studies. Both antibody sequences and plasmid DNA are ready to be transferred. If none of the preselected candidate antibodies works for you, our DimAb® B cell library can easily obtain more than 10,000 positive hits in as short as a month's time.

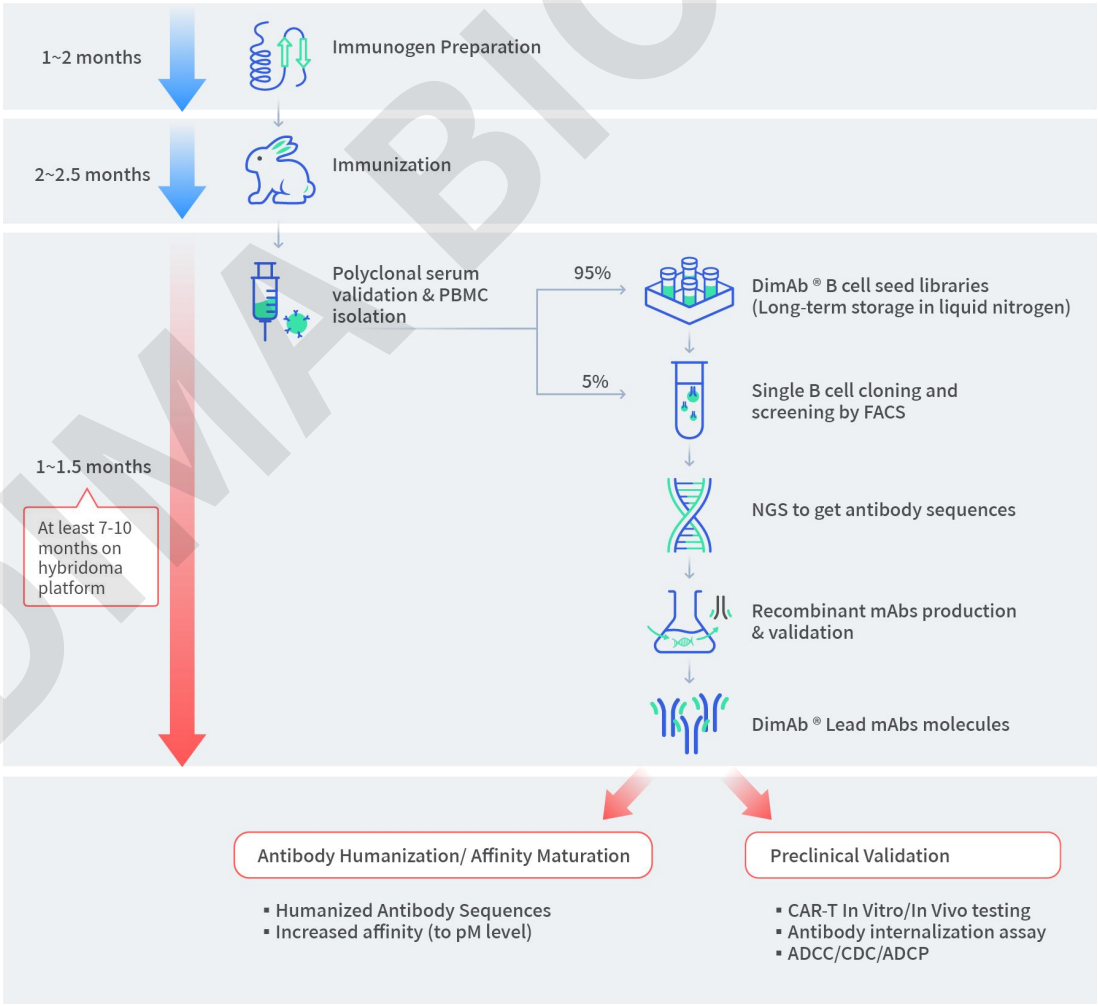


Figure 4: Development of DimAb® B cell seed libraries and lead mAbs molecules

DimAb® lead mAb molecules >>

- Pre-developed lead mAb molecules with defined functional evaluation data
- Next day delivery of lead mAb molecules
- Guaranteed performance without big upfront cost
- Flexible terms to meet different business expectations
- No binder no payment, zero risk
- Save customer at least 8 months in development time

DimAb® B cell seed libraries >>

- Live B cells stored and pre-validated with functional screening
- From cultivation to IgG sequences in 30-45 days
- High diversity and large number of unexplored binders
- No binder no payment, zero risk
- Save customer at least 6 months in development time

DimAb® lead mAb molecules

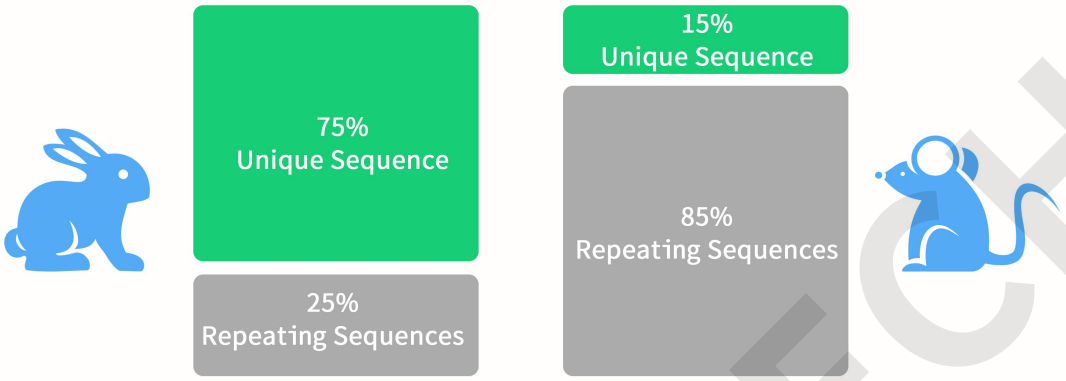
Using our proprietary single B cell antibody development platform, we have completed hit-to-lead discovery for around 300 druggable targets and obtained over 5000 antibody molecules with IgG sequence information and antibody validation data. The complete list of our available targets can be found on our website (www.dimabio.com/lead-antibody-molecule).

We choose rabbits as our host animals because rabbits have a high success rate with challenging antigens like small peptides or post-translational modification (PTM) sites, which are often non-immunogenic in mice. The antibodies produced in rabbits also recognize more epitopes per antigen than mouse antibodies. Rabbit antibodies have better affinities and specificities than mouse antibodies. They can bind to target proteins in the picomolar range, while mouse antibodies generally recognize proteins to the nanomolar range. The druggability of rabbit antibodies has also been verified in 2019. As of today, there are 2 FDA approved drugs based on rabbit antibodies.

Three Advantages of Rabbit Monoclonal Antibodies

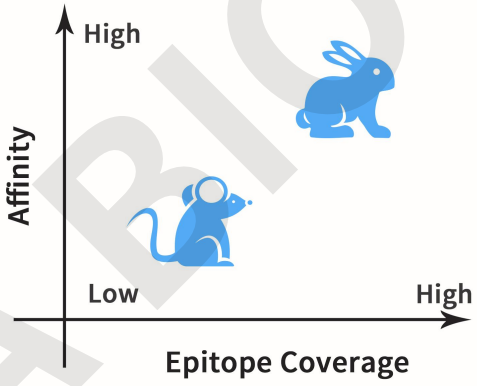
Rabbit monoclonal antibody has three advantages in antibody drug discovery.

Ontogeny of rabbit B-cell and antibody repertoires



Weber J,et al.,Experimental & Molecular Medicine,2017

High Affinity & Broad Epitope Coverage

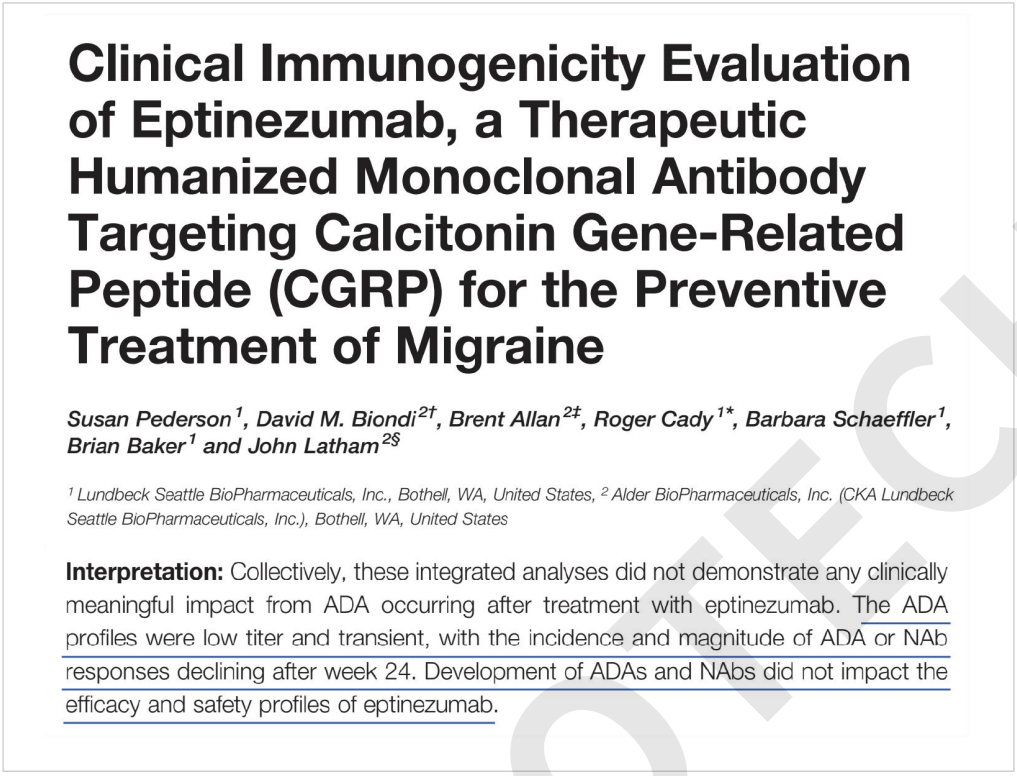


Zhu WM,(Unpublished data)

The druggability of rabbit antibodies has been verified

A treatment for wet age-relatedmacular degeneration(wet AMD)
FDA approved in 2019

A CGRP blocker for migraine prevention
FDA approved in 2020



Clinical Immunogenicity Evaluation of Eptinezumab, a Therapeutic Humanized Monoclonal Antibody Targeting Calcitonin Gene-Related Peptide (CGRP) for the Preventive Treatment of Migraine

Susan Pederson[1], David M. Biondi[2†], Brent Allan[2‡], Roger Cady[1], Barbara Schaeffler[1], Brian Baker[1] and John Latham[2§]*

[1] Lundbeck Seattle BioPharmaceuticals, Inc., Bothell, WA, United States, [2] Alder BioPharmaceuticals, Inc. (CKA Lundbeck Seattle BioPharmaceuticals, Inc.), Bothell, WA, United States

Interpretation: Collectively, these integrated analyses did not demonstrate any clinically meaningful impact from ADA occurring after treatment with eptinezumab. The ADA profiles were low titer and transient, with the incidence and magnitude of ADA or NAb responses declining after week 24. Development of ADAs and NAbs did not impact the efficacy and safety profiles of eptinezumab.

DimAb® B cell seed libraries

DimAb® B cell seed libraries are a pool of frozen B cells prepared from immunized rabbits, and pre-validated by FACS as positive binders to antigen proteins. Most of the libraries have been used for the screening of lead mAbs by our internal R&D team and customers. The antibodies prepared from DimAb® B cell seed libraries have shown diverse CDR sequences and good druggability in downstream testing results. Each library contains lots of potential binders for antibody drug discovery.

Key Features:

- Pre-made B cell libraries containing diverse FACS binders for target protein
- Quick freezing in liquid nitrogen after PBMC isolation to prevent injury to cells and keep the diversity of original B cells.
- Risk free, no binder no payment
- Save your time for lead molecule discovery, skip immunization and early screening steps (3~4 months).
- Two steps validation:
  - Validation of immunized rabbit serum as specific binders to the target proteins.
  - Validation of capabilities for FACS binder screening during lead mAbs discovery.

Advantages of DimAb® Lead Antibody Program

1 **Quality**

All antibodies are FC validated

2 **Sequence**

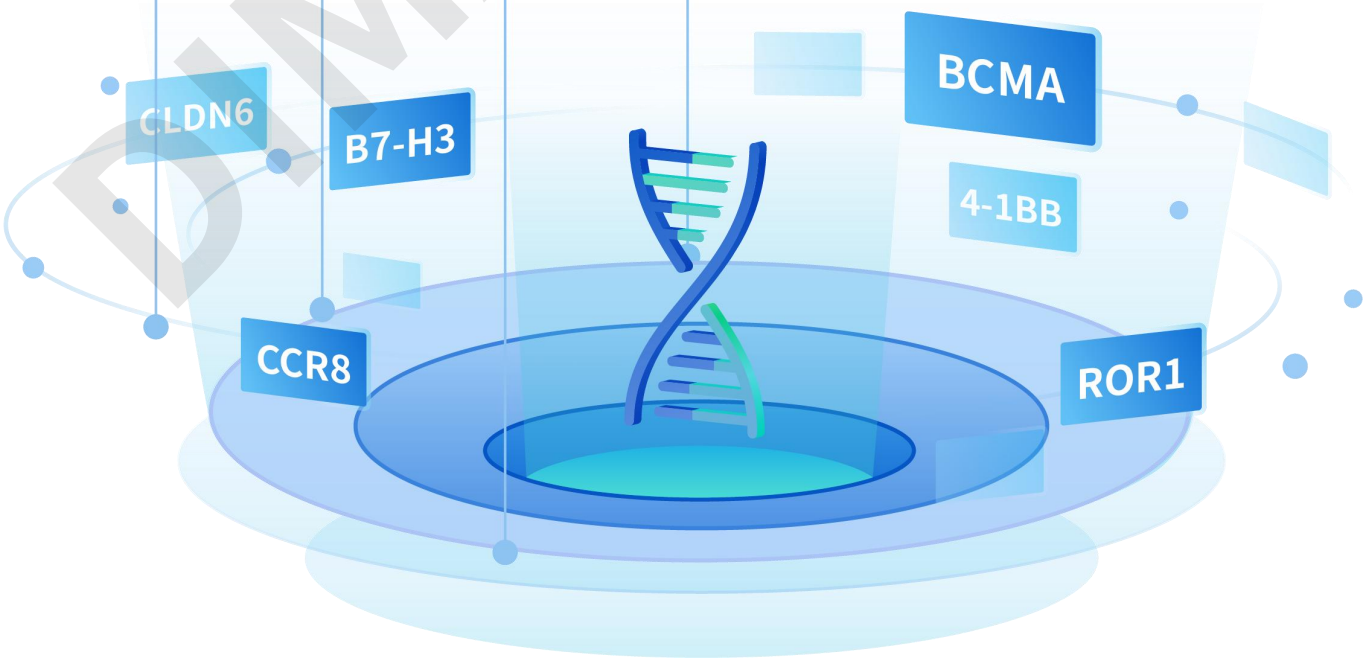
We ensure unique sequence of CDR region, and customers can buy out all B cells produced from their immunized animal.

3 **Worry-free**

Provide professional technical support and high-quality antibody production services

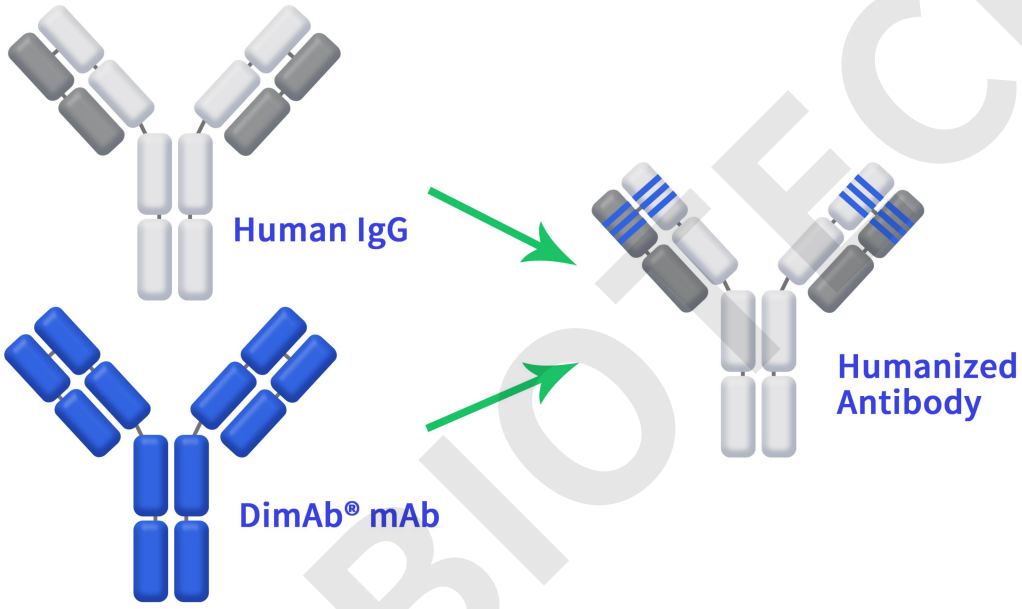
4 **More Choices**

We have an average of more than 5 pre-stocked and pre-validated monoclonal antibody molecules for each target, which are free for testing. If you need more molecules, our DimAb® B cell library can help you easily obtain more than 10,000 positive hits in as short as a month's time.

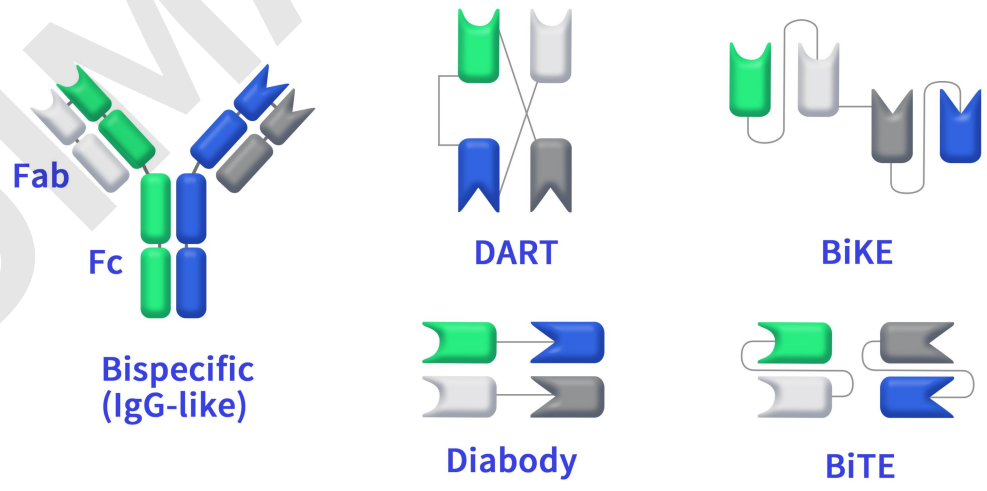


Antibody Engineering

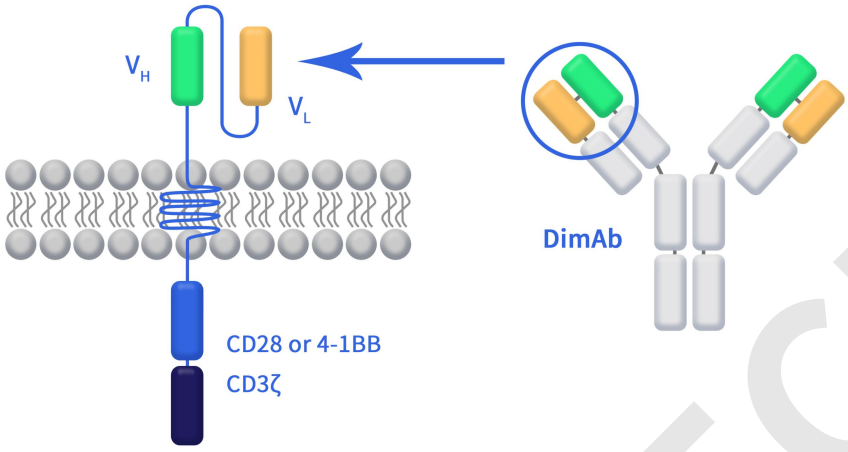
In addition to on-shelf pre-stocked and pre-validated hit-to-lead stage antibody molecules, DIMA offers multiple antibody engineering platforms to provide researchers with more powerful bullet for their antibody drug discovery.



Antibody Humanization & Affinity Maturation Platform



Bi-specific Antibody Platform



CAR-T in-vitro and in-vivo testings

Antibody Humanization & Affinity Maturation

Antibody humanization is important for therapeutic antibody development, especially for the candidate antibodies derived from animal sources. DIMA Biotech has developed a proprietary mammalian display platform for antibody humanization and affinity maturation, DiLibrary™ antibody engineering platform. With this platform, we can deliver a panel of humanized variants with improved affinity than its parental antibody. In addition to improved affinity, the engineered clones also exhibit improved developability for downstream development, such as high expression level and low aggregation tendency. Therefore, DiLibrary™ system is a superior antibody engineering platform to help us optimize antibody molecules with better developability.

1. Mammalian cell display
2. Experiment oriented screening
3. Antibody yield assessment
4. At least 3x increase in affinity
5. Finish in 48 days

Advantages:

Powerful
One-step process for humanization, optimization & developability assessment

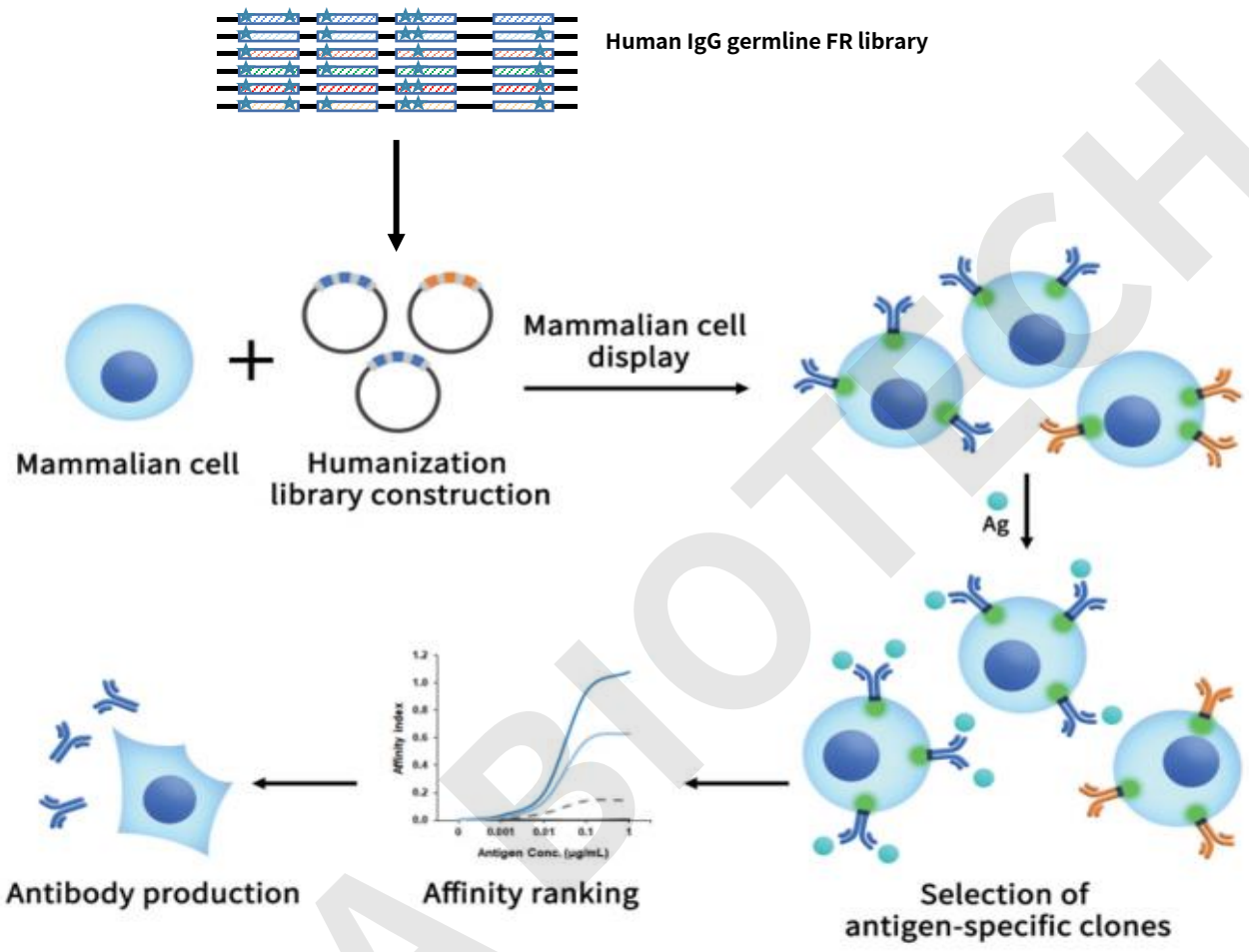
Flexible
Custom designed mAb library for your needs

Fast
Deliver humanized mAb sequences and data in 48 days.

Complete
One stop shop from antibody discovery , optimization to functional evaluation.

Risk Free
Guarantee affinity after humanization

Platform Workflow



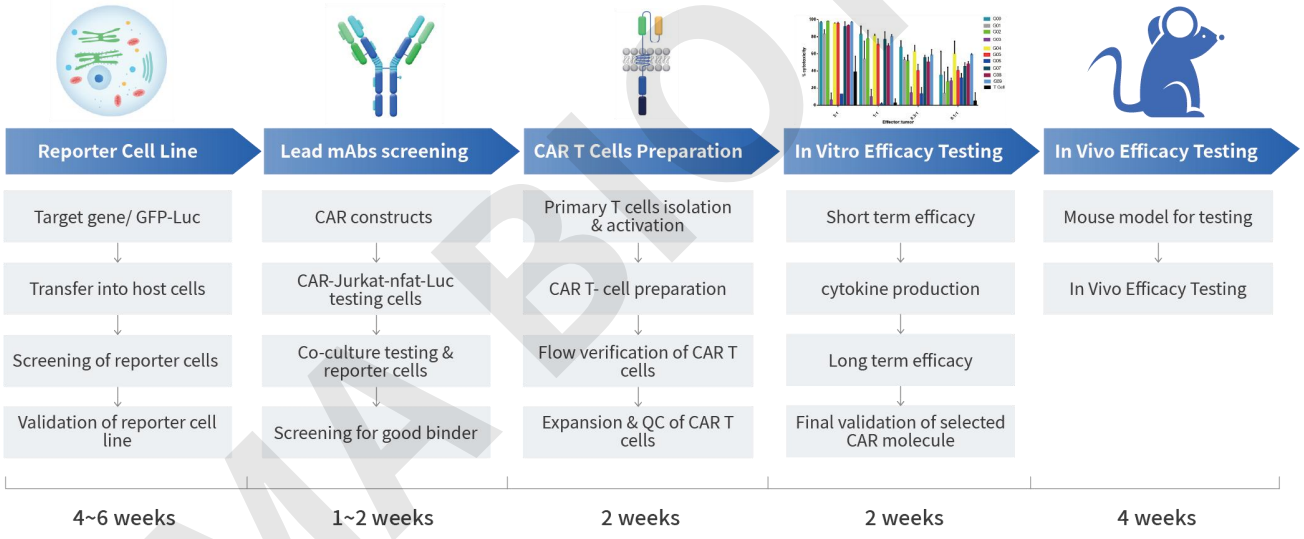
Services & Deliverables

Service	Deliverable	Time
Construct humanized antibody expression library	Progress Report - Validation of antibody expression on cell membrane - Validation of antibody-antigen Flow cytometry analysis	1~2 weeks
Antibody affinity screening and ranking	Progress Report - Affinity ranking result	2~3 weeks
Antibody cloning and sequencing	Progress Report - DNA cloning status	2~3 weeks
Antibody production and affinity testing	QC report for selected humanized antibodies - antibody sequences - FACS binding - SPR examination	2 weeks
PTM removal	Antibody sequence after PTM removal PTM removal report	Varies

CAR-T Lead Antibody Molecules

A good lead antibody molecule is crucial to ensure the efficacy and safety of CAR-T cells in clinical applications. DIMA Biotech has established a complete screening platform for CAR-T lead antibody molecules. We have a range of advanced technologies, in vitro and in vivo assays to identify and optimize the best candidate antibody molecules for CAR-T therapy. Our approach has been successful in developing good CAR-T lead molecules for various cancer targets. We are constantly working to expand our capabilities and bring more innovative solutions to the field of cancer immunotherapy. As of today, we have developed 5000+ pre-stocked and pre-validated mAb molecules against 300+ drug targets. Some of them have already entered clinical trial phase.

Workflow



Platform Advantages

On-shelf Availability

5000+ mAb molecules for 300+ druggable targets with validation data and antibody sequences

Fast Turnaround

A complete platform for CAR T cell preparation, and ready-to-use CAR-T and reporter cells to save the assay preparation time.

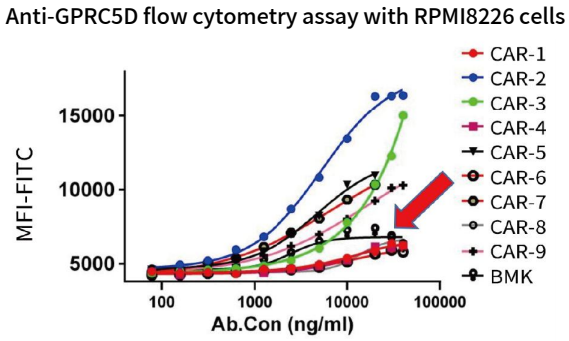
High Quality

Verified CAR molecules with In-Vitro and In-Vivo data and antibody sequences.

CAR Targets Under Development

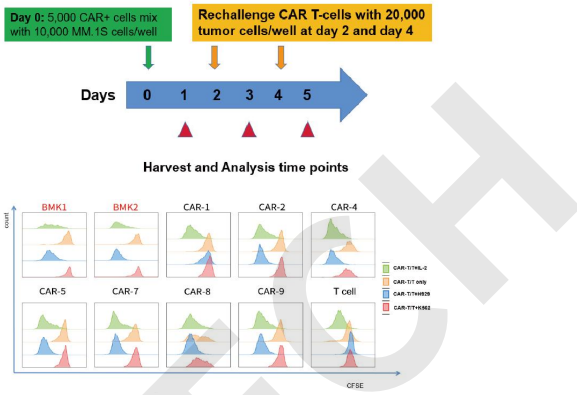
Target	Lead mAb discovery	CAR Construction	Lentivirus packaging	In Vitro Testing	In Vivo Testing	IIT
GPRC5D						
BCMA						
GPRC5D&BCMA						
CD138						
GPC3						
FcRL5						
Claudin18.2						
CD38						
Mesothelin						
5T4						
CD70						
AXL						
CD123						
MUC1						
EGFR						
CEACAM5						
CS1						
FAP						
B7H3						
EpCAM						
ROR1						
GUCY2C						
FOLR1						
CD5						
CD7						
CDH17						
CD79A						
CD79B						
CD30						
CD33						

Case Studies

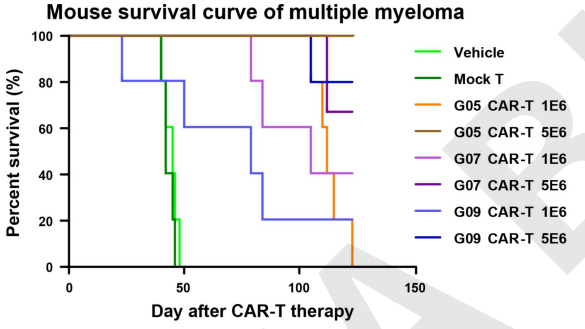


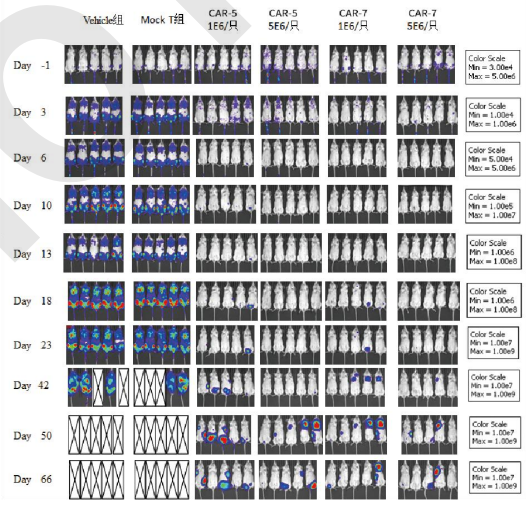
The red arrow indicates the reference antibody which is in clinical trial

Flow analysis for CAR molecule affinity



Characterization of CAR T cell expansion and cancer cell killing efficacy during In Vitro assay

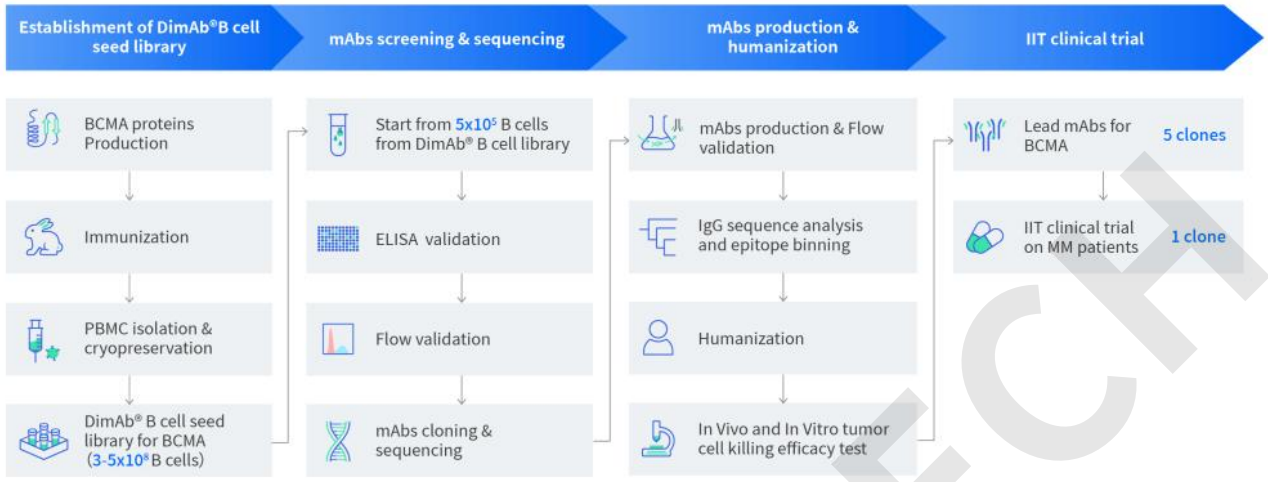




In Vivo efficacy experiment in mouse

Case Study: Development of anti-BCMA therapeutic mAbs for CAR-T application

Multiple myeloma (MM) is the second most common hematologic malignancy after non-Hodgkin lymphoma with a very high probability of recurrence. It is considered as an incurable disease, and the development of new treatment options is urgent. BCMA is an ideal target for the treatment of multiple myeloma (MM) because it is highly expressed in MM cells but not other normal cells except for mature B lymphocytes and plasma cells.

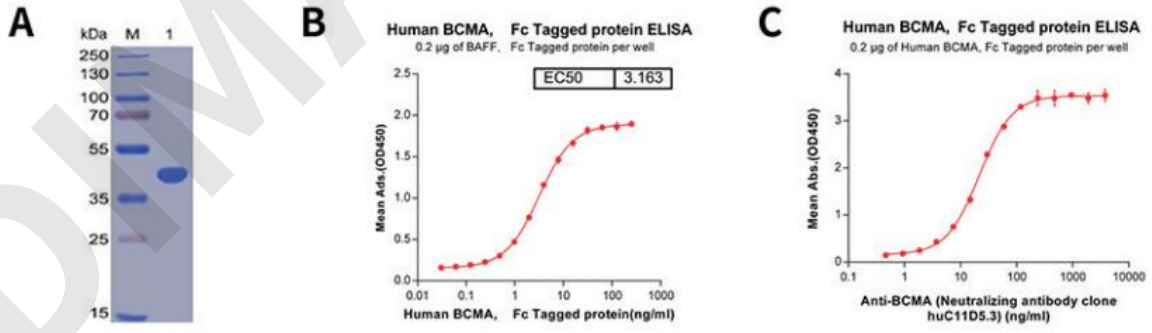


Workflow for BCMA DimAb® lead antibody molecules project

Equipped with our recombinant protein production and single B cell antibody development platforms, we developed 5 lead mAbs molecules for human BCMA targets with verified functional data and antibody sequences. We also created a DimAb B cell seed library for the human BCMA target which presumably contains 10 thousands Flow positive BCMA binders and provides a good resource for additional screening of anti-BCMA therapeutic lead mAb molecules.

Antigen Preparation: Bioactive BCMA Recombinant Protein Preparation

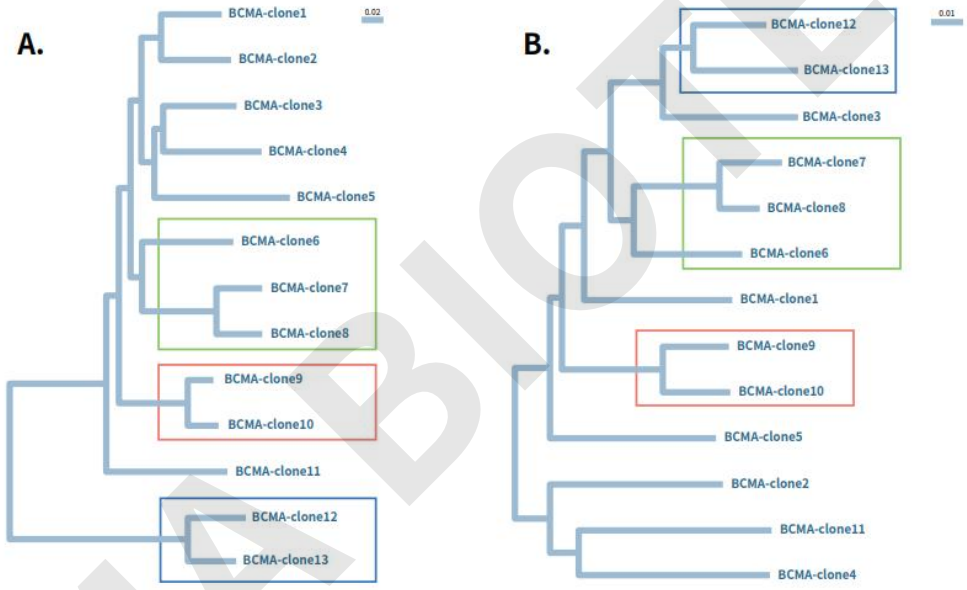
To ensure good functional property and native structure of immunogen, the purities and activities of purified proteins were validated by SDS-PAGE and different binding assays before immunization.



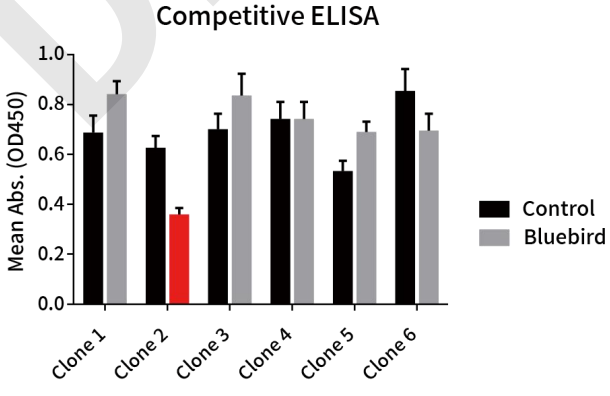
BCMA Antigen Validation Data: A. Human BCMA, Fc-tagged on SDS-PAGE under reducing condition. B. ELISA plate pre-coated by 2 μg/ml (100 μl/well) Human BAFF, hFc tagged protein can bind Human BCMA, Fc- tagged protein in a linear range of 0.03-15.625 ng/ml. C. ELISA plate pre-coated by 2 μg/ml (100 μl/well) Human BCMA, Fc-tagged protein can bind Anti-BCMA (huC11D5.3) (Its variable region was used to construct scFv portion of CAR-T Idecabtagene vicleucel (bb2121).) in a linear range of 3.71-22.29 ng/ml

Lead mAbs Development against BCMA proteins

After immunization, around 3~5 X10^8 B cell pools were isolated from the immunized rabbit and quickly frozen in liquid nitrogen as our B-cell seed library for human BCMA target. From 4ml of rabbit whole blood, we identified 70 ELISA positive B cell clones, out of which 13 worked for the flow application and were picked up for the downstream mAb cloning and sequencing. After epitope comparison, 5 new clones were identified with unique CDR sequences comparing to the Bluebird anti-BCMA huC11D5.3 clone. All of them have shown comparable tumor cell killing efficacy with the Bluebird anti-BCMA huC11D5.3 clone in the CAR-T application after humanization. One has been picked up for an Investigator Initiated trial (IIT). The preliminary data is encouraging at this moment.

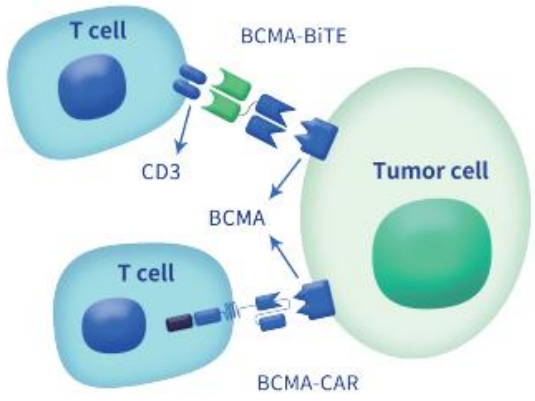


Phylogenetic analysis of 13 different Anti-BCMA DimAb clones A) heavy chain and B) Light chain. All these clones work for flow application. The boxed regions indicate heavy and light chains of the same clone come from the same lineage group.



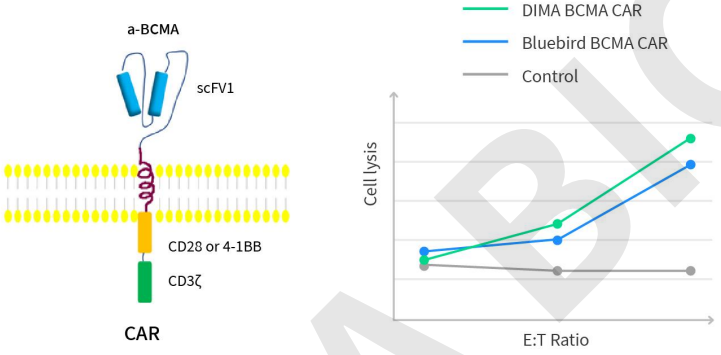
Epitope comparison between different anti-BCMA DimAb clones and anti-BCMA huC11D5.3 clone (Bluebird bb2121). ELISA plate was coated with recombinant BCMA-hFc fusion protein, followed by pre-blocking with huC11D5.3 antibody (Grey bar) or rabbit control IgG (Black bar) and then different rabbit DimAbs antibodies were added to check the competitive inhibition of huC11D5.3. One clone exhibits the strongest inhibition (Red bar). This data indicated that one clone binds to the same epitope as bb2121.

Antibody Engineering



Based on the Anti-BCMA antibody gene sequence provided by the DimAb® recombinant monoclonal antibody platform, we humanized five validated high-affinity clones and constructed bispecific BITE molecules, CAR molecules for the in vitro functional verification.

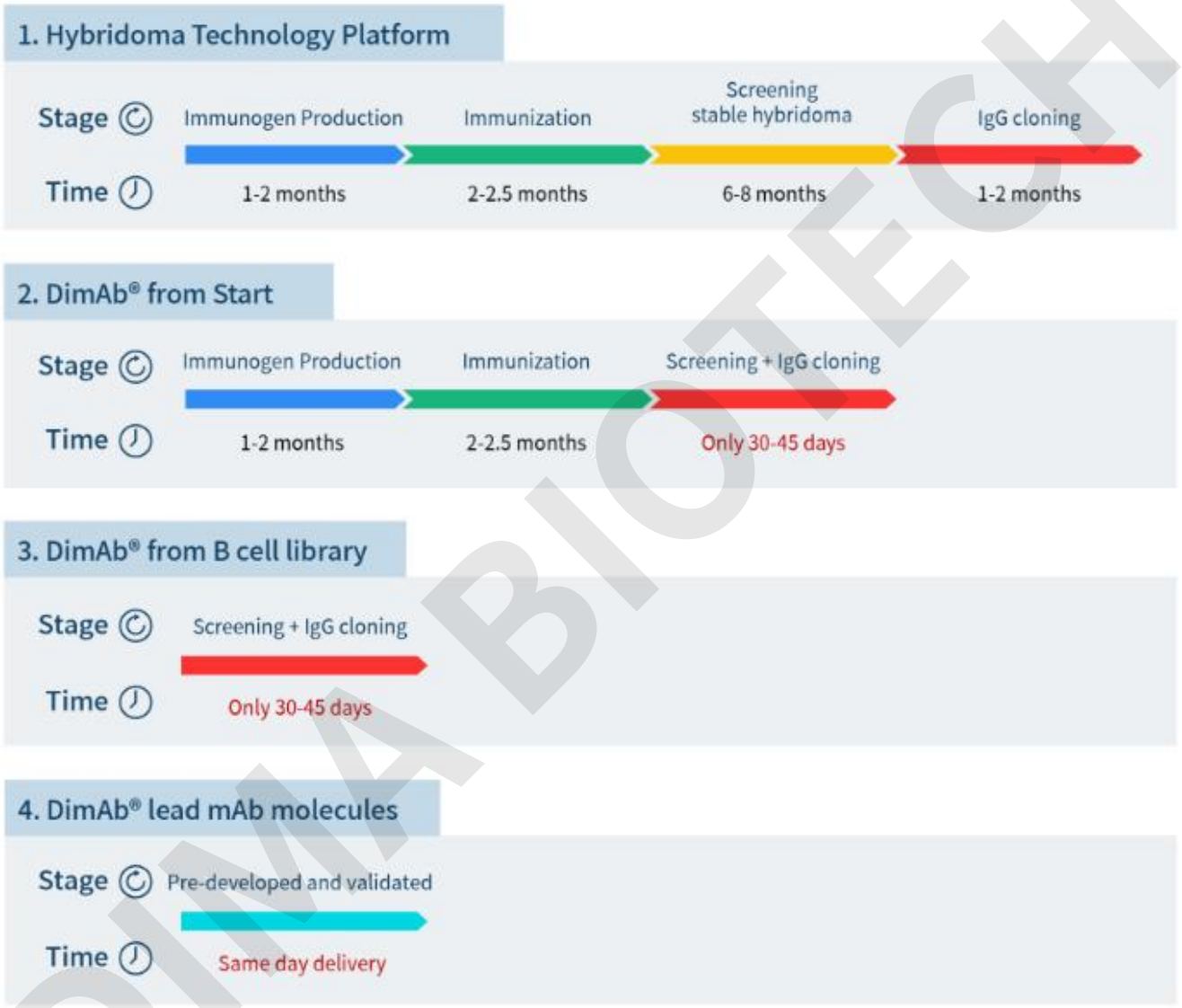
Development of anti-BCMA therapeutic mAbs for CAR-T application



Perform in vitro and in vivo efficacy verification analysis of 5 humanized modified CAR targeting BCMA . The test results show that our molecules are not inferior to Bluebird's antibody under different target ratio conditions, whether in vitro or in vivo tests.

Flexible Business Models

We provide flexible business models for the lead antibody molecule development. Customers can select the ideal molecules from our pre-stocked and pre-validated antibodies and test them for free in their own assays. If the results meet customers requirement, they can sign an agreement to directly obtain the sequence of the required antibody and corresponding intellectual property. If there is no ideal molecules in the existing antibody list, we can screen more molecules from our DimAb® B cell seed libraries and it only takes 1~2 month for us to get more positive candidates for further testing. For targets without B-cell libraries, we provide customized development services using our well-established antigen preparation platform and single B-cell antibody development platform. The time required for each models is shown in next page, compared with the traditional hybridoma platform, DIMA Bio can save at least 6~8 months of antibody development time.



DIMA Biotechnology LTD

Dedicate on immuno-oncology，
Perfect with recombinant mAb development

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